Acta Veterinaria Hungarica 47 (4), pp. 409–418 (1999)

USE OF PROTECTED METHIONINE (MEPRON M 85) IN CATTLE

J. SCHMIDT*, P. SIPŐCZ, Éva CENKVÁRI and J. SIPŐCZ

Department of Animal Nutrition, Pannon Agricultural University,
H-9201 Mosonmagyaróvár, Vár 4, Hungary

(Received March 22, 1999, accepted June 24, 1999)

The ruminal stability of Mepron M 85 and the effect of supplementation with Mepron M 85 on free methionine level of blood were studied in rumen-fistulated cows and rumen- and duodenum-fistulated growing bulls. In five rumen-fistulated cows *in situ* 69.5% and 64.6% of the methionine content of Mepron M 85 was found after ruminal incubation of 16 h and 24 h, respectively. Daily rations of the rumen-fistulated cows were supplemented with 15.0 g DL-methionine and 17.7 g Mepron M 85, which increased the free methionine level of blood from 13.64 μmol/L to 15.35 and 20.46 μmol/L, respectively, three hours after feeding. In the four rumen- and duodenum-fistulated growing bulls, supplementation with 15.0 g DL-methionine and 17.7 g Mepron M 85 increased the total methionine getting into the duodenum during 24 h from 14.99 g to 16.84 and 20.84 g, respectively. The influence of Mepron M 85 on milk production was studied in 35 pairs of Hungarian Fleckvieh × Holstein-Friesian cows. The animals were coupled on the basis of the number of finished lactations, milk production in the previous lactation, and the date of calving. Daily supplementation of 18.0 g Mepron M 85 increased daily milk production significantly ($p < 0.05$), by 1.24 litres. Milk fat content also increased significantly (from 3.10% to 3.19%, $p < 0.05$) in the experimental group. The supplementation did not influence milk protein content.

Key words: Protected methionine, Mepron M 85, bypass methionine

Microbial protein synthesis in the rumen is of essential importance in the protein and amino acid supply of ruminants, since microbial protein may meet 65–85% of the protein requirement depending on the production level (Schiemann, 1981). Its precondition is the perfect energy supply of animals, since microbial synthesis is a highly energy-demanding process. According to Hagemeister and Kaufmann (1979), 100 g organic matter provides energy for the synthesis of 22 g microbial protein in the rumen. In the nutrient requirements published by ARC (1980) it is supposed that 18.75 g microbial protein is produced from 100 g digestible organic matter (DOM). The new Hungarian protein evaluation system (Schmidt et al., 1998) considers that 100 g fermentable organic matter (FOM) is sufficient for the synthesis of 160 g microbial protein.

*E-mail: schmidt@movar.pate.hu

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It is known that microbial protein includes less methionine than casein, therefore several scientists consider methionine as the first limiting amino acid for microbial protein synthesis (Fischer, 1972; Schelling et al., 1973; Broderick et al., 1974; Clark, 1975; Schwab et al., 1976; Illg et al., 1987).

In the first third of lactation of high-yielding dairy cows, it often occurs that the energy requirement is not met and that decreases microbial protein production in the rumen. This, together with insufficient methionine supply, can result in the limitation of milk production. Experimental results, which show that DL-methionine administered directly into the abomasum or small intestine and methionine products of low rumen degradability can increase milk production of cows, are explainable (Rogers and McLeay, 1977; Daugaard, 1978; Kaufmann and Lüpping, 1979; Lettner, 1983; Burgstaller et al., 1983*a*; Leibetseder et al., 1985).

Based upon these facts, some experiments using a bypass methionine product, Mepron M 85, were performed to answer the following questions: (*i*) How stable is the methionine product Mepron M 85 in the rumen and how much of it can escape from the rumen without degradation? (*ii*) What kind of effect does Mepron M 85 have on the free methionine level of the blood plasma? (*iii*) How does Mepron M 85 influence the milk production of dairy cows in the first third of lactation?

Materials and methods

Mepron M 85 is a bypass methionine product which contains 85% DL-methionine encapsulated (manufactured by Degussa AG, Hanau, Germany). The ruminal stability of Mepron M 85 was examined *in situ* in 5 rumen-fistulated dairy cows. The cows were Hungarian Fleckvieh × Holstein Friesian R_4 crosses and were in the last third of lactation. Bags made of Scrynel with a pore diameter of 40 μm were used. The incubation periods were 1, 2, 4, 8, 12, 16 and 24 h. The ruminal stability of the methionine product was checked three times for each incubation time. After incubation, each bag was washed 8 times for 10 min with cold tap-water to remove residues of rumen juice. Water was changed after each washing period. Since Mepron M 85 contains only free DL-methionine (and no protein), the methionine left in the bags after incubation was determined with a iodometric method (Degussa Analytik/Analysis, 1986).

The effect of Mepron M 85 on free methionine level of the blood plasma was studied in a periodical trial using 5 dairy cows. The trial included one control and two experimental periods. Each period took 14 days: pre-feeding was of 9 days duration and the measurement period lasted 5 days. Blood samples were taken daily from the jugular vein 3 h after feeding, as in our experience the methionine content of the blood plasma reaches its peak in the 3rd hour after feeding. Free methionine content of the blood plasma was determined by ion-

exchange column chromatography using an Aminochrom II amino acid analyser, after protein removal with 60% perchloric acid, neutralisation with KOH, centrifugation, and uptake in citrate buffer.

Composition of the daily ration of cows:	
Maize silage	20.0 kg
Alfalfa hay	6.0 kg
Compound	5.0 kg

Composition of the compound:		*Nutrient content of the daily ration:*	
Maize	50.0%	Dry matter	14.5 kg
Wheat	41.0%	NE_l	98.1 MJ
Soybean meal	4.0%	Crude protein	2100.3 g
Vitamin and minerals premix	5.0%	Methionine	35.6 g
Total	100.0%	Cystine	27.0 g
		Crude fibre	3006.6 h
		Crude fibre in dry matter	20.7%
		Ca	135.0
		P	93.6

In experimental periods 1 and 2, 15.0 g DL-methionine and 17.7 g Mepron M 85, respectively, was mixed to the above ration. In the 1st and 2nd phase of the experiment, the DL-methionine and the Mepron M 85 supplements were mixed into the cows' daily ration and consumed fully by the animals.

The rumen stability of Mepron M 85 was examined also in a periodical trial with 4 rumen- and duodenum-fistulated growing bulls of 550 ± 12 kg average body weight. The bulls were Hungarian Fleckvieh × Holstein Friesian R_4 crosses. The trial included one control and two experimental periods. In the two experimental periods, DL-methionine and Mepron M 85 was added to the diet of animals, respectively. Each period lasted for 14 days: pre-feeding was of 11 days duration and the measuring period lasted 3 days. On the first and third days of the measuring period, duodenal chymus was collected for 24 h. During these periods, the quantity of chymus passing through the duodenum in 24 h was measured via re-entrant cannulas and aliquot samples were taken.

Total methionine content of the chymus was determined by hydrolysis with 6 M hydrochloric acid in a microwave equipment (Milestone MLS-1200 MEGA). The time of hydrolysis was 16 min and the periods were 1 min at 250 W and, after a 1-min interval, 5 min at 250 W and at 400 W, respectively, and 4 min at 650 W.

Based on the methionine content of samples and the quantity of chymus passing through the duodenum, the amount of methionine getting into the duodenum during 24 h was calculated.

Composition of the daily ration of growing bulls:			
Grass hay	4.5 kg		
Compound	3.8 kg		
Composition of the compound:		*Nutrient content of the daily ration:*	
Maize	77.00%	Dry matter	7.2 kg
Wheat	11.00%	NE_m	48.8 MJ
Extracted sunflower meal	9.75%	NE_g	30.3 MJ
Feed lime	1.25%	Crude protein	911.0 g
Feed salt	0.50%	Methionine	13.6 g
Vitamin and minerals premix	0.50%	Cystine	10.8 g
Total	100.00%	Ca	37.0 g
		P	24.1 g

In the experimental periods 1 and 2, 15.0 g DL-methionine and 17.7 g Mepron M 85, respectively, was added to the compound.

The effect of Mepron M 85 on milk production was studied with 35 Hungarian Fleckvieh × Holstein-Friesian cow pairs. The cows were kept in groups. When forming cow pairs, the following aspects were considered: (*i*) Number of finished lactations; (*ii*) milk production in the previous lactation; (*iii*) Days from calving until the start of experiment; (*iv*) Holstein-Friesian gene ratio; (*v*) milk production at the beginning of the trial.

The parameters listed above were as follows:

	Experimental group	Control group
Number of the finished lactations	2.0	2.0
Milk production in the previous lactation, kg	7715	7764
Days from calving until the start of experiment	33.3	35.5
Holstein-Friesian gene ratio, %	94.8	95.0
Milk production at the beginning of trial, kg/day	37.0	37.2

The two groups were fed the daily rations shown in Table 1.

Table 1

Composition and nutrient content of the daily rations of dairy cows

		Experimental group	Control group
Maize silage	kg	17.50	17.50
Alfalfa haylage	kg	3.00	2.90
Green alfalfa	kg	8.40	8.40
Alfalfa hay	kg	4.00	4.00
Brewer's grains, wet	kg	7.00	7.00
Barley meal	kg	7.30	7.30
Premix	kg	0.13	0.13
Protein compound	kg	2.15	2.12
Nutrient content of the daily rations:			
Dry matter	kg	23.79	23.74
NE_l	MJ	157.68	157.31
Crude protein	g	4136.60	4118.60
Methionine	g	57.30	57.20
Cystine	g	48.70	48.60
Crude fibre	g	4406.60	4396.30
Crude fibre in dry matter	%	18.50	18.50
Ca	g	172.10	171.30
P	g	85.80	85.80

Composition of the protein compound: soybean meal 91.5%, MCP (mono-calcium phosphate) 2.0%, feed lime ($Ca\ CO_3$) 3.0%, salt 2.5%, vitamin and minerals premix 1.0%; total: 100.0%

Cows in the experimental group were fed 18.0 g Mepron M 85 daily.

The milk production of each group was measured daily and milk composition was determined on the basis of pooled milk of the groups twice a week for 75 days.

Statistical analysis of data was performed by the program Statistica of StatSoft, Inc. (2325 East 13th Street, Tulsa, OK 74104, USA).

Results

The results of *in situ* experiments with Mepron M 85 are shown in Table 2.

It can be concluded that methionine loss grew relatively fast in the first 4 h of incubation and then degradation of Mepron M 85 in the rumen slowed down. Almost half (49.6%) of the total degradation was measured in the first 4 h of incubation. After 24 h of incubation, 64.6% of the original methionine content was found in Mepron M 85. In reality, probably a larger amount of methionine leaves the rumen without degradation, since feedstuffs do not stay in the rumen for 24 h in the case of intensive feeding. In general, protected methionine is fed

to high-yielding dairy cows, especially in the first third of lactation when 8% of the rumen content escapes per hour because of intensive feeding, so the daily ration stays in the rumen for 12–13 h in average. Therefore, 78–79% of the methionine content of Mepron M 85 escapes from the rumen without degradation.

Table 2

Weight and methionine loss of Mepron M 85 in the rumen

Incubation time, hours	Weight loss, %	Methionine loss, %
1	8.75 ± 0.43	7.15 ± 0.44
2	11.00 ± 0.60	11.71 ± 0.71
4	13.28 ± 0.93	17.57 ± 1.40
8	16.47 ± 0.99	18.14 ± 1.45
12	20.29 ± 1.52	21.67 ± 1.90
16	26.44 ± 1.27	30.55 ± 2.91
24	29.33 ± 2.35	35.40 ± 3.57

There are various data in the literature about the rumen-stability of different bypass methionine products. In the experiments of Kaufmann and Lüpping (1982), 50–90% of the methionine content of the studied bypass methionine products was degraded in the rumen. According to the results of Mate (1985), only 20% of methionine was degraded in the case of hydroxy-methyl-methionine (HMM-Ca) and Ketionin.

The free methionine level of the blood plasma showed that the methionine content of Mepron M 85 indeed escaped from the rumen without degradation and that the coat surrounding methionine granules decomposes in the abomasum and small intestine. Supplementation with 15.0 g DL-methionine and 17.7 g Mepron M 85 increased the free methionine level of the blood plasma from 13.64 μmol/L in the control period to 15.35 μmol/L and 20.84 μmol/L, respectively. In the case of Mepron M 85, the methionine increment of 50% was found to be significant ($p < 0.01$). Like in this study, supplementation with bypass methionine (HMM-Ca) increased free methionine content of the blood in the experiments of Koch and Tanner (1981) and Spiekers (1988) as well. Hagemeister (1984) found that feeding 45 g bypass methionine increased free methionine content of blood at the same rate as did 15 g DL-methionine infused into the abomasum.

The assumption about the strong protection of Mepron M 85 is supported also by the results of trials conducted with growing cattle (Table 3). When the daily ration was supplemented with 15 g DL-methionine, the amount of methionine getting into the duodenum increased by 1.85 g (12.3%) in 24 h as compared to the control period. This showed that methionine was not degraded completely in the rumen. Kaufmann and Lüpping (1982) also found that 95% of DL-methionine supplementation became

degraded in the rumen. Addition of 17.7 g Mepron increased the quantity of methionine getting into the small intestine by 5.85 g (39.0%), which increase was significant. It is less than that was suspected on the basis of the *in situ* results.

Table 3

Effect of addition of DL-methionine and Mepron M 85

Period	Dry matter getting into the duodenum g/24 h	Methionine content of chymus g/kg dry matter	Methionine getting into the duodenum g/24 h
Control	4745.08 ± 1875.6	3.16 ± 0.22	14.99 ± 5.61
DL-methionine	4784.06 ± 1762.7	3.52 ± 0.39	16.84 ± 5.51
Mepron M 85	4974.94 ± 1575.6	4.19 ± 0.38	20.84 ± 4.97

This can be explained by the following factors: (*i*) In growing cattle, as a result of the less intensive feeding only 4–5%, rather than 8%, of the feed escaped from the rumen per hour. Therefore, the feed stayed in the rumen for 20–24 h instead of 12–13 h, which increased methionine loss from the bypass product. (*ii*) It is possible that in the first part of the duodenum (at the site of the cannula) only a part of the protected methionine was present in the chymus because the coating of the M 85 granule had not been fully decomposed in the abomasum. Although the difference between methionine losses, detected *in situ* and *in vivo,* can be explained mainly by the less intensive feeding of growing cattle, the second factor cannot be excluded either.

Our results coincide with those of Langar et al. (1978) who found 9–54% of methionine in the duodenum when bypass methionine (n-stearoyl-DL-methionine) was fed, depending on the type and dose of product.

The results of the field trial are shown in Table 4. It can be concluded that during the 75 days of the trial 18 g Mepron M 85 increased daily milk production of the experimental group by 1.24 kg on the average ($p < 0.05$). These results coincide with the findings of other authors. Increase of milk production has been reported as an effect of supplementation with Mepron (Kaufmann and Lüpping, 1979; Leibetseder et al., 1985; Günther and Hagena, 1987; Spiekers, 1988). Feeding of other bypass methionine products also increased milk production (Journet and Hoden, 1980; Sprondly, 1981; Küther, 1982; Yang et al., 1986).

Although fat content of milk was higher in the experimental group than in the control one, the difference did not prove to be significant. At the same time, some authors reported an increase of milk fat content after addition of bypass methionine (Burgstaller et al., 1983*a*; Günther and Hagena, 1987). Feeding of Mepron M 85 did not influence milk protein content. Because of higher milk produc-

tion, milk fat and milk protein production also increased in the experimental group. For milk fat, the increase was significant ($p < 0.05$).

There are some reports on the increase of milk fat content as the effect of feeding bypass methionine (Burgstaller, 1983*a, b*; Hagena, 1985; Lettner, 1983). In a previous experiment conducted by us, feeding of HMM-Ca increased not only daily milk fat production but milk fat content as well (Schmidt et al., 1987).

Table 4

Effect of Mepron M 85 on milk production and milk composition

		Experimental group	Control group
Milk production	kg	$34.93^{a} \pm 2.25$	$33.69^{b} \pm 2.38$
Milk composition			
Dry matter	%	11.23 ± 0.37	11.15 ± 0.44
Fat	%	3.19 ± 0.19	3.10 ± 0.23
Protein	%	2.96 ± 0.10	2.96 ± 0.10
Daily production by milk			
Dry matter	kg/day	3.94 ± 0.20	3.78 ± 0.21
Fat	kg/day	$1.12^{a} \pm 0.06$	$1.05^{b} \pm 0.07$
Protein	kg/day	1.04 ± 0.05	1.00 ± 0.05

a, b = values with different superscripts within rows are significantly different ($p < 0.05$)

Some scientists (Chandler et al., 1976; Buttery and Foulds, 1985; Lundquist et al., 1985*b*) explain the increase of milk fat production by the beneficial effect exerted by bypass methionine on the activity of rumen microbes. According to other researchers (Chamberlain and Thomas, 1982; Huber et al., 1984), higher milk fat production may be due to the availability of more methionine for intermediary metabolism, which positively influences liver activity and fat metabolism.

The beneficial effects of bypass methionine can be explained by different reasons. The first of these should be the classical amino acid effect, namely that the feeding of methionine enhances protein synthesis. Beyond that, some scientists have established that methionine addition has a beneficial effect on the activity of rumen microbes (Lundquist et al., 1985*a*; Patterson and Kung, 1988), increases the number of protozoa (Vuyst et al., 1975; Günther and Hagena, 1987). Methionine also has a positive effect on lipoprotein metabolism (Koch, 1984; Doil, 1985).

It can be concluded that the bypass methionine product Mepron M 85 has satisfactory ruminal stability and, as a result of its feeding, the quantity of methionine getting into the small intestine increases. As the methionine supply of cows improves, milk and milk fat production increases significantly in the first third of lactation.

Acknowledgement

The authors express their special thanks to Degussa AG for supporting the experiments.

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